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The relationship between Anti-Mullerian Hormone and ovarian response and fertility outcome in infertile women undergoing assisted reproductive techniques (ART)

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ABSTRACT

Background and Aim: Anti-Mullerian Hormone (AMH) is an indicator of the number and quality of eggs produced during the menstrual cycle, which is related to the number of antral follicles, and the outcome of assisted reproductive techniques. The aim of this study was to evaluate the relationship between anti-mullerian hormone levels and ovarian response and pregnancy outcomes in infertile women undergoing assisted reproductive techniques (ART). *Materials and Methods:* This cross-sectional study was performed on 186 infertile women who underwent assisted reproductive techniques (ART) referred to Besat Hospital Infertility Center, *from 2016 to 2020*. Demographic information, FSH level, AMH level, number of gonadotropin injections, number of antral follicles and number of fetuses were evaluated. Also, pregnancy outcomes including abortion, fetal death, fetal malformation and birth were determined and recorded in a questionnaire. *Results:* The mean of anti-mullerian hormone level was 3.9 (0.1 to 12 ng / ml) and the mean of FSH level was 6.1 (0.6 to 12.7 IUI / L). A considerable association was observed between anti-mullerian hormone level with the number of ovarian follicles, number of injected HMG, number of eggs, number of total embryos ($P < 0.05$). There was no significant relationship between anti-mullerian hormone levels with abortion, fetal death, live birth and neonatal malformations ($P > 0.05$). *Conclusion:* Determination of AMH levels can help for administration of gonadotropins, prediction of the antral follicle numbers, the number of eggs and the quantity of embryos obtained.

Keywords: Anti-mullerian hormone, IVF, Pregnancy outcome, Gonadotropin, Agonist

1. INTRODUCTION

Clinical knowledge and technological advancement have considerably improved the outcomes of assisted reproductive techniques, but a major factor in fertility success is the number and quality of oocytes produced by the ovaries following hormonal stimulation (Ubaldi et al., 2014). An important limiting factor in the success of IVF is the poor ovarian response, which is reported in 10 to 15% of women (Jirge et al., 2014). Ovarian reserve reflects the function of the ovary and also the number and condition of eggs within an ovary (Fauser et al., 2008). Ovarian reserve reflects the growing follicle, and ultimately the number of eggs which is associated with IVF outcomes. Low ovarian reserve is defined by an abnormal increase in FSH, less than 4 small antral follicles in the ovaries, and a decrease in anti-mullerian hormone (AMH) (Barad et al., 2007; Barad et al., 2011). All three factors are associated with low egg and embryo and ultimately impaired pregnancy and the possibility of live birth (Lukaszuk et al., 2013).

Ovarian reserve is assessed by different methods. The quantity of antral follicles (AFC) has been suggested as a useful test in determining ovarian reserve. Other ways to tests ovarian reserve include determining FSH level, serum inhibin B level and AMH (Broekmans et al., 2005; Chang et al., 1998; Hendriks et al., 2005; Chang et al., 2010). The optimum method for determining the ovarian reserve is to measure the level of AMH, and considering its sensitivity and specificity, it is equivalent to AFC, and is better than FSH, estradiol, inhibin B (Chang et al., 2010). FSH, inhibin B and estradiol are hypersensitive in the early stages of ovarian reserve depletion; however, serum AMH levels are independent of the menstrual cycle and are not altered by GnRH agonists or oral contraceptives (Li et al., 2013).

AMH is one of the members of TGF_B large family, which is secreted by the small follicles granulosa cells to regulate the early stages of follicular growth. AMH is clinically used to predict ovarian hyperstimulation syndrome, determining ovarian reserve, and the quality of residual oocytes. Since AMH is a hormone that is produced by growing follicles, it is an indicator of the number and quality of eggs produced during the menstrual cycle (Fanchin et al., 2011; Ocal et al., 2005). It also has a good relationship with female age, number of AFCs and the result of assisted reproductive techniques (Li et al., 2013). Therefore, it is considered as the most accurate biomarker of aging and ovarian reserve (Nikolaou et al., 2004). Recent studies have reported an association among AMH levels and pregnancy outcome. High AMH was associated with an increased rate of abortion and lower live births (Gleicher et al., 2016; Goswami et al., 2017). While Moreau et al., (2019) showed that in IUI cycles the rate of abortion and live birth was not related to AMH levels.

Although measurements of anti-molar hormone levels are now widely used to predict the response to ovarian stimulation before in vitro fertilization, there is disagreement about the incidence and prediction of pregnancy outcome. Therefore, the objective of this research was to evaluate the association among anti-mullerian hormone levels and ovarian response and pregnancy outcomes in infertile women.

2. MATERIALS AND METHODS

This cross-sectional (descriptive-analytical) study was performed on 186 infertile women who underwent assisted reproductive techniques (ART) referred to Besat Hospital Infertility Center, Sanandaj, Iran, *from 2016 to 2020*. Exclusion criteria included: no liver, kidney and blood disease, no signs of polycystic ovary syndrome on ultrasound, no complications of assisted reproductive technology, no hormonal therapy at least one month ago and no male infertility.

The demographic information including age, body mass index, history of infertility, cause of infertility, history of abortion and number of pregnancies were obtained using the medical files of the patients and recorded in a questionnaire. Also, FSH level obtained by radioimmunoassay and AMH level obtained by ELISA method were extracted from patients' medical records and recorded in a questionnaire. The number of gonadotropin and clomiphene injections and the quantity of ovarian follicles, recovered eggs, type and number of embryos were also determined. In case of pregnancy in the studied women, the consequences of pregnancy including: abortion, fetal death, fetal malformation and live birth were recorded in a questionnaire.

Clinical pregnancy was considered as one or more pregnancy sacs that can be seen by ultrasound. Abortion was considered as any spontaneous or treatment pregnancy loss during clinical pregnancy. Live birth was contemplated as the parturition of at least one healthy new-born with a gestational age equal to or greater than 7 months (Chen et al., 2016). This study has been authorized by the ethics committee of Kurdistan University of Medical Sciences with the code (IR.MUK.REC.1399.237).

Data Analysis

Data were analyzed using SPSS.22 software. Data were summarized using descriptive indicators such as mean, standard deviation, frequency and relative frequency. Normality of quantitative dependent variables; were evaluated using Kolmogorov-Smirnov test.

Chi-square and one-way analysis of variance were used to analyze the relationships between variables. Pearson correlation test was implemented for determining the correlation between anti-mullerian hormone and FSH.

3. RESULTS

Out of 186 infertile women, 109 women (58.6%) had primary infertility and 77 (41.4%) had secondary infertility. The mean of anti-mullerian hormone was 4.0 ± 3.0 (range of 0.1 to 12 ng / ml) and the mean of FSH was 6.1 ± 2.5 (range of 0.6 to 12.7 IUI / L). Among participants, 58.6% had never been pregnant and 7.5% were pregnant four or more times. A total of 68.3% of women had no records of abortion and 15.1% had a history of more than one abortion (Table 1).

Table 1 Description of variables in participants

Variables		Standard deviation ± mean	Range
Age		34.1±5.5	20-45
BMI		27.8±4.1	17.7-38.2
Duration of infertility		6.1±4.7	1-24
AMH		4.0±3.0	0.1-12
FSH		6.1±2.5	0.6-12.7
		No.(%)	
Parity	No pregnancy	109(58.6)	
	1-3	63(33.9)	
	4 and more	14(7.5)	
History of abortion	No abortion	127(68.3)	
	1	31(16.6)	
	2 and more	28(15.1)	
Reproductive assistance method	IVF agonist	146(78.5)	
	IVF antagonistic	40(21.5)	

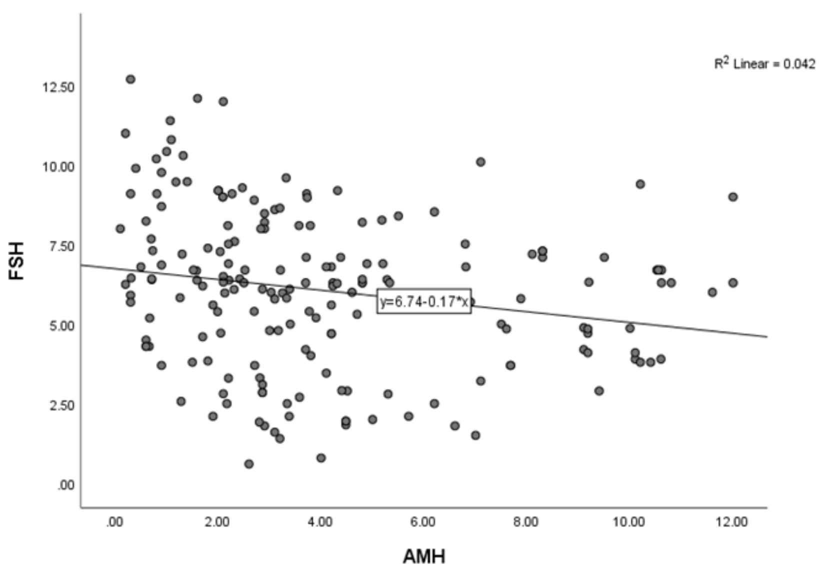

Figure 1 Correlation between anti-mullerian hormone and FSH in women

A significant inverse correlation was observed between anti-mullerian hormone levels and FSH (r = - .20 and p = .005) (Figure 1). There was no statistically significant relationship between anti-mullerian hormone level and the quantity of ovarian follicles. No considerable association was found among anti-mullerian hormone levels and the number of injected FSH in women undergoing IVF (p = 0.07). There was a statistically significant relationship between anti-mullerian hormone levels with the number of injected HMG (p = 0.001). Also, a considerable association was observed among the number of M2 oocytes in IVF agonist women and the level of anti-murine hormone (p = 0.0001). No considerable association was found among M1, GV and degenerated oocytes with antimullerian hormone levels (p> 0.05). There was a significant relationship between the total number of fetuses, type A and type B fetuses and the level of anti-mullerian hormone in IVF agonists women (p = 0.0001), but in IVF antagonist women, no considerable association was observed (p> 0.05) (Table 2).

Table 2 the relationship between anti-mullerian hormone levels and ovarian response in women

Variables	Anti-mullerian hormone level ng / dl	<1	1-3	>3	P value
The ovarian follicles	16 and more Follicles, right ovary (number (%))	10(14.7)	18(26.5)	40(58.8)	
	16 and more Follicles, left ovary (number (%))	8(10.9)	17(23.3)	48(65.8)	0.55
Injected gonadotropin	Mean FSH injected	17.3±12.5	13.3±7.5	12.4±5.7	0.013
	Mean HMG injected	9.7±7.3	9.1±7.5	6.3±7.0	0.023
Number of recovered eggs	IVF agonist	3.4±2.0	7.1±4.9	10.2±4.4	0.0001
	IVF antagonistic	4.0±1.7	5.1±2.7	6.9±3.7	0.1
M2	IVF agonist	2.4±1.4	5.8±4.3	8.7±4.2	0.0001
	IVF antagonistic	3.3±1.3	4.5±2.5	6.3±3.3	0.6
M1	IVF agonist	1.3±0.7	2.1±1.4	2.3±3.3	0.24
	IVF antagonistic	1±0	1±0	1.4±0.5	0.21
GV	IVF agonist	0	2±0.7	0.9±0.2	0.2
	IVF antagonistic	0	1±0	1.5±0.7	0.32
Degenerated	IVF agonist	0.4±0.2	0.8±0.2	0.7±0.2	0.44
	IVF antagonistic	1±0	0	1±0	0.99
Total fetuses	IVF agonist	2.2±1.9	4.6±2.5	8.0±3.9	0.0001
	IVF antagonistic	3.9±1.6	4.3±2.7	4.9±2.9	0.69
Type A fetuses	IVF agonist	1.7±1.5	3.8±2.1	6.8±3.3	0.0001
	IVF antagonistic	3.4±1.2	3.7±2.0	4.2±2.6	0.85
Type B fetuses	IVF agonist	0.5±0.7	0.8±1.1	1.2±1.3	0.046
	IVF antagonistic	0.5±0.5	0.6±1.3	0.8±0.9	0.52

There was no considerable association among anti-mullerian hormone levels in IVF agonist and IVF antagonist groups with abortion, fetal death, birth status and neonatal malformations (p> 0.05) (Table 3).

Table 3 the relationship between anti-mullerian hormone levels and pregnancy outcomes in women

Variables	Anti-mullerian hormone level ng / dl		<1	1-3	>3	P value
Abortion	IVF agonist	Yes	4(8.2)	19(38.8)	26(53.1)	0.44
		No	15(15.5)	31(32)	51(52.5)	

	IVF antagonistic	Yes	2(12.5)	7(43.8)	7(43.8)	0.1
		No	6(25)	3(12.5)	15(62.5)	
Fetal death	IVF agonist	Yes	1(9.1)	3(27.3)	7(63.3)	0.9
		No	18(13.3)	47(34.8)	70(51.9)	
	IVF antagonistic	Yes	1(20)	1(20)	3(60)	0.99
		No	7(20)	9(25.7)	19(54.3)	
Live birth	IVF agonist	Yes	14(16.1)	28(32.2)	45(51.7)	0.39
		No	5(8.5)	22(37.3)	32(54.2)	
	IVF antagonistic	Yes	5(26.3)	2(10.5)	12(63.2)	0.14
		No	3(14.3)	8(38.1)	10(47.6)	
Neonatal abnormalities		Yes	1(16.7)	2(33.3)	3(50)	0.85 Fisher
		No	7(14)	13(26)	30(60)	

4. DISCUSSION

Due to the exclusive production of anti-mullerian hormone in adult women, this hormone can be used for assessing the ovarian activity (Anderson et al., 2012). In our study, the mean of anti-mullerian hormone was 3.2 (range 0.1-12), in a study by Geralyn et al., (2016) in the 40-31 years' age group the mean of AMH was 2.41 (range 0.15-14.5) and in a study by Li et al., (2013) mean was 2.48 (range of 1 to 38.5 ng / mL). Compare to other studies the mean of AMH level was higher in our study. Our findings showed that with increasing levels of anti-mullerian hormone, the number of gonadotropins injected in women undergoing IVF was reduced and was inversely related. In the study by Li et al., (2013) also there was a significant correlation between anti-mullerian hormone and injected gonadotropin dose. In IVF, the role of serum AMH measurement in predicting ovarian response to gonadotropin stimulation, specifying therapies to improve efficacy and safety, and ultimately predicting overall treatment success has been confirmed (Iliodromiti et al., 2014).

The findings of this study showed a direct relationship between the level of anti-mullerian hormone and the quantity of AFCs, and increasing the level of anti-mullerian hormone, the quantity of AFCs had increased. Using the same dose of FSH for all patients, Freiesleben et al., (2010) showed that the quantity of mature follicles (18 mm) depended on AMH levels. Ficicioglu et al., (2006) reported that AMH levels reflected the size of the antral follicle reservoir and concluded that AMH levels can be implemented as a biomarker to determine the number of eggs to be collected by ovarian stimulation. In a study by Aydin et al., (2015) the quantity of AFCs was significantly higher in the group with higher AMH level, which is consistent with our findings.

In our study, the number of recovered eggs was directly related to the increase in anti-mullerian hormone levels, so that with increasing hormone levels, the number of recovered eggs, especially in IVI agonist women, was significantly higher, which was due to the presence of M2 type eggs. A relationship between baseline serum AMH levels of the follicular phase and the quantity of oocytes after the induction of ovulation was found by Seifer et al., (2006). In particular, they collected higher numbers of eggs and more mature oocytes in patients with higher serum AMH levels. The results of Mirza Moradi et al., (2017) showed that for one-unit increase in the logarithm of blood level of AMH, the chance of releasing one egg will increase by 24%. In a study by Tehranizadeh et al., (2016) the quantity of AFCs was associated with the number of eggs and the number of fresh and frozen embryos.

In a study by Patrelli et al., (2012) there was a correlation between AMH and the quantity of eggs. In a study by Zhang et al., (2019) also the levels of AMH were positively associated with the quantity of recovered eggs in both young and old groups. Ganidou et al., (2015) showed that markers of mother's age, AMH, and FSH variables could well and accurately predict ovarian excessive response. Hamdine et al., (2015) showed that the use of AMH level had a high accuracy in predicting ovarian responses, with the difference that this accuracy was more for excessive ovarian response than poor ovarian response. In a study by Moradi et al., (2017) the predictive accuracy was for poor ovarian response. The quantity of recovered eggs is a strong marker for clinical success (Drakopoulos et al., 2015). This may be due to an increase in the number of quality embryos, which increases the frequency of embryo transfer.

Our findings showed that the quantity of embryos had a direct and significant relationship with the level of anti-mullerian hormone in the subjects in the IVF agonist group, but in the IVF antagonist group, although the number of embryos increased, but it was not significant. Various studies have reported higher AMH in women who have had a clinical pregnancy (Wang et al., 2015; Dondik et al., 2017). Some studies have reported moderate (Rhoades et al., 2009) or non-significant (Tremellen et al., 2010) correlations between AHM levels and chance of pregnancy. The reason for this difference can be due to the sample size and the selected statistical population. In our study, no considerable association was found among anti-mullerian hormone levels and fetal death and abortion; however, the frequency of abortion was higher in women with anti-mullerian hormone level higher than 3 ng / ml, so that with the increase in the level of anti-mullerian hormone, the frequency of abortion increased.

In a study by Vikas et al., (2019) low anti-mullerian hormone levels had no relationship with the rate of live birth after intrauterine insemination and abortion rates. However, in a study which conducted by Glincher et al., (2019) to determine the relationship between FSH levels, AMH and embryo count in patients with good, moderate and poor prognosis, high AMH was related to an increment in abortion rate at all ages. Low AMH, regardless of age, was observed to be associated with increased pregnancy loss; the reason for this difference may be due to higher fetal aneuploidy. In this study, no considerable association was found between anti-mullerian hormone levels and live birth and it was almost "similar" in all three levels. However, in a study by Li et al., (2013) those women who had a live birth in the first cycle, the AMH serum level was significantly higher than those who failed to give live birth.

In a study by Moreau et al., (2019) anti-mullerian hormone levels had no significant relationship with the rate of live births. In a study by Goswami et al., (2017) the mean level of AMH in the group with live birth was higher than the group without live birth. Live births were less likely in the younger age group. Older women with higher AMH levels had significantly more live births than their peers with lower AMH level. In a study by Gleicher et al., (2016) AMH was unexpectedly associated with pregnancy and labor outcomes even for cases more than 43 years of age. Several other studies have reported a significant association between higher AMH and live birth rates (Bakas et al., 2015; Moro et al., 2016; Ganidou et al., 2014). In a study, AFC, AMH and FSH were not associated with total clinical pregnancy (Tehraninezhad et al., 2016). Therefore, it is necessary to avoid unnecessary anxiety in women of childbearing age, especially young novices due to low levels of AMH (Zhang et al., 2019).

In the present study a significant inverse correlation was observed between anti-mullerian hormone and FSH, that is, FSH decreased with increasing anti-mullerian hormone. However, this inverse correlation was not very strong. In a research by Li et al., (2013) anti-mullerian hormone had a significant inverse correlation with FSH, which is consistent with our findings. Decreased AMH levels do not indicate a decrease in normal fertility in young or old women. Therefore, caution should be exercised in the proper use of AMH assessments, especially in pre-pregnancy counselling to avoid excessive and unnecessary fertility anxiety (Chenxi et al., 2021).

5. CONCLUSION

Determination of AMH levels can be helpful in administering gonadotropins, predicting the quantity of antral follicles, recovered eggs, and the number of fetuses obtained. But anti-mullerian hormone levels had no association with pregnancy outcomes.

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Conflicts of interest

The authors declare that there are no conflicts of interests.

Data and materials availability

All data associated with this study are present in the paper.

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